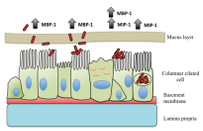
Virulence



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Trehalose metabolism: a sweet spot for *Burkholderia pseudomallei* virulence

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Trehalose, trehalase, *Burkholderia pseudomallei*, stress, biofilm, virulence

Trehalose is a sugar composed of two α-D-glucose molecules linked by an α, α-1,1- glucoside bond which is produced by a variety of organisms such as bacteria, archeae, fungi, plants and invertebrates [1]. Vertebrates including humans are unable to synthesize trehalose. However, they retained the ability to break down the sugar into glucose by producing the enzyme trehalase. Analogous to glycogen which is a major reserve compound produced by animals, yeast and bacteria, trehalose was initially considered to solely act as a carbon and energy storage molecule. It is for example the main sugar in the hemolymph of insects where it is used as a source of energy during flying and it is also used as a storage carbohydrate in spores and resting cells of fungi. The past decades and years of research on trehalose considerably changed the picture of the restricted function of trehalose and that of its precursor trehalose-6-P (T6P). Trehalose is now recognized as a biomolecule with unique properties that serves highly diverse functions for prokaryotic and eukaryotic cells ranging from cellular protectant to a factor that shapes symbiotic and pathogenic host interactions. In plants, it seems that these functions of trehalose are taken over by sucrose but T6P is now considered as a new plant growth regulator involved in many aspects of plant development [2].

Five distinct pathways for the synthesis of trehalose have been described, all of which are found in bacteria and two of which are present in eukaryotic organisms [1]. The TPS/TPP (OtsA/OtsB in bacteria) pathway is the most conserved biosynthesis route present in all three domains in the tree of life. It forms trehalose by the action of trehalose-6-phosphate synthase (TPS/OtsA) which synthesizes T6P from glucose and glucose 6 phosphate. Trehalose-6-phosphate phosphatase (TPP/OtsB) subsequently dephosphorylates T6P to trehalose. In addition to glucose and derivatives thereof maltose and maltodextrins are utilized by bacteria and archeae to synthesize trehalose involving the TS or TreY/TreZ pathway, respectively.

A remarkable feature of trehalose is its ability to enable a variety of organisms to endure extreme physiological stresses such as dehydration, high salt concentrations, heat and freezing. The sugar is accumulated to high levels in anhydrobiotic plants, fungi and bacteria that tolerate

complete desiccation and resume metabolic activity upon rehydration. Trehalose is the major compatible solute with protective effects at high osmolarity. Accumulation of endogenous trehalose in response to salt stress enables *E. coli* to sustain a concentration of 0.5 M NaCl [3]. Furthermore, the sugar is necessary for *E. coli* and other bacteria to survive heat and low temperatures [4]. The physicochemical properties of trehalose make the compound an exceptional protectant of cells, organs, biomaterials, proteins and lipid bilayers at both high and low temperatures used for cryostorage. Trehalose is a chemically inert sugar which is very stable at low pH and extreme temperatures forming non-hygroscopic crystals. Several mechanisms underlying the efficient stabilization of biomolecules by trehalose are currently discussed including glass formation and water retention or water replacement.

Plants such as *Arabidopsis thaliana* that produce barely detectable levels of trehalose display increased drought tolerance upon overexpression of trehalose biosynthesis genes [2]. The main reason for the increased drought stress tolerance in these cases was not the increase in trehalose, of which the concentration is still very low, but probably the increase in T6P. This was recently confirmed using *A. thaliana* trehalase mutants where, counter intuitively, it was shown that overexpression of trehalase results in better drought stress tolerance. In such transgenic lines trehalose levels were lower than in the wild type plants, but there was a small increase in T6P levels, which in plants seem to be much more important as a regulator of stress tolerance [5, 6].

Several bacterial and fungal species can become pathogens of humans. The human host defends itself against pathogens through the innate and adaptive immune system. An important aspect of the innate immune system is the oxidative burst in neutrophils, macrophages and dendritic cells which kills most pathogens. The protective effect of trehalose accumulation in microorganisms against reactive oxygen species has raised the question of the general role of trehalose in protecting pathogens from the hostile environment of the host during infection [7].

Accumulating evidence supports an important and complex role of trehalose metabolism in symbiosis and virulence. Trehalose biosynthesis genes of the mycorrhizal fungus *Amanita*

muscaria are upregulated during symbiosis and bean plants inoculated with *Rhizobium etli* overexpressing the trehalose biosynthesis gene *otsA* displayed higher numbers of nodules, nitrogenase activity and increased yields [8]. With respect to virulence, an ABC transporter involved in trehalose recycling of *Mycobacterium tuberculosis* is required for full virulence and one of the three trehalose biosynthesis pathways present in *M. tuberculosis* is implicated in the development of chronic infections [9]. Abrogation of trehalose synthesis in *Pseudomonas aeruginosa* PA14 results in a strong attenuation of virulence in plant leaves but not in mice and nematode models of infection [10]. Likewise, trehalose production is an essential virulence trait in pathogenic fungi. In *Cryptococcus gattii* for instance, deletion of *TPS1* and *TPS2* genes has a strong impact on capsule production, protein secretion and survival in mice and nematodes. Deletion of the *TPS2* gene in *C. albicans* shows attenuation of virulence in a mouse model for systemic infection [11]. A hypervirulent phenotype is also observed in *Cryptococcus neoformans* upon deletion of the gene *NTH2* encoding a trehalase [12]. The intricate interplay of virulence and trehalose metabolism was further illustrated by the finding that a *Fusarium graminearum TPS1* mutant is impaired in mycotoxin production [13]. Finally, trehalose itself can act as signaling molecule that stimulates expression of host defense genes in Arabidopsis [14].

In this issue of *Virulence* Vanaporn et al. [15] addressed the role of trehalose metabolism in stress adaptation and virulence of *Burkholderia pseudomallei*, the causative agent of the disease melioidosis in humans and animals. Clinical presentations of melioidosis range from more benign chronic and localized infections to fulminant septicemia [16]. *B. pseudomallei* is found in the soil in tropical areas and was shown to tolerate salt, temperature and pH stress conditions. Moreover, the bacteria are capable of intracellular survival in professional phagocytes and amoeba [17]. Apart from common virulence factors such as flagella, capsule, type III, V and VI secretion systems, the large genome of the *B. pseudomallei* (7.2 Mbp) is predicted to encode a variety of metabolic pathways many of which have not been investigated for their role in infection. Orthologs of *otsA*, *otsB* and the trehalase gene *treA* are present in *B. pseudomallei*

indicating that the bacteria are able to synthesize and catabolize trehalose. Vanaporn et al. investigated the impact of trehalase on stress adaptation and virulence in *B. pseudomallei* which has not been studied before in bacteria. The authors first showed that growth of a *B. pseudomallei treA* deletion mutant was not affected in M9 minimal medium using glucose as a carbon source compared with the wild type K96243. However, in M9 medium supplemented with trehalose as the sole carbon source the *treA* mutant was unable to replicate indicating that TreA is functional and active during growth in culture medium. The deletion of *treA* increased tolerance against heat and cold stress but reduced the ability of *B. pseudomallei* to form biofilms and to proliferate in J774.A1 macrophages. Furthermore, the authors identified TreA as a decisive factor in determining the outcome of an infection: In the absence of TreA virulence of *B. pseudomallei* was dramatically reduced leading to 100% survival of mice after intraperitoneal challenge in comparison with 100% lethality at the same time point after infection with the wild type. Whether the deletion of *treA* has an impact on the level of endogenous trehalose remains to be determined in future studies. At present, potential explanations for the loss of virulence of the Δ*treA* mutant are its inability to degrade trehalose into glucose or the inhibition of virulence factors by higher levels of trehalose. Such a phenotype was also observed for *C. albicans* where high intracellular trehalose levels result in a strong reduction in the body temperature induced elongated growth phenotype [18]. Collectively, the study by Vanaporn et al. shows that trehalose homeostasis deserves more research into its role in virulence of *B. pseudomallei* and other pathogenic bacteria.

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